

What is claimed is:

1. A heteropolymer composition comprising: at least three different amino acids selected from the group of amino acids consisting of the class of aromatic amino acids, the class of charged amino acids, and the class of aliphatic amino acids, the selected  
5 amino acids being randomly polymerized in a linear configuration, the composition having therapeutic activity in a subject suffering from an autoimmune disease, excluding the combination of amino acids tyrosine, glutamic acid, lysine, and alanine.
2. A heteropolymer composition according to claim 1, wherein the class of  
10 aromatic amino acids comprises tyrosine and phenylalanine.
3. A heteropolymer composition according to claim 2, wherein the class of charged amino acids comprises arginine, lysine, glutamic acid and aspartic acid.
- 15 4. A heteropolymer composition according to claim 3, wherein the class of aliphatic amino acids comprises glycine, proline, valine, leucine, isoleucine, and alanine.
5. A composition according to claim 4, comprising: the amino acids tyrosine, alanine and lysine.  
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6. A composition according to claim 5, comprising: the amino acids polymerized in a molar ratio of at least 3 moles of lysine per mole of tyrosine and at least 4 moles of alanine per mole of tyrosine.
- 25 7. A composition according to claim 6, comprising: the amino acids tyrosine, lysine, and alanine polymerized in the molar ratio of 1.0:4.0:5.0.
8. A composition according to claim 4, comprising: the amino acids tyrosine, glutamic acid, and lysine.

9. A composition according to claim 4, comprising: the amino acids valine, alanine and lysine.

10. A composition according to claim 4, comprising: the amino acids  
5 phenylalanine, alanine and lysine.

11. A therapeutic composition, comprising: a heteropolymer having the components of at least one amino acid of each of charged amino acids, aliphatic amino acids, and aromatic amino acids, excluding the combination of amino acids tyrosine,  
10 glutamic acid, lysine, and alanine, the therapeutic composition being in a pharmaceutically acceptable carrier, for treatment of a subject having an autoimmune disease.

12. A therapeutic composition according to claim 7, wherein the autoimmune disease is selected from the group consisting of: multiple sclerosis, myasthenia gravis,  
15 Hashimoto's disease, systemic lupus erythematosus, uveitis, Guillain-Barre' syndrome, Grave's disease, idiopathic myxedema, autoimmune oophoritis, chronic immune thrombocytopenic purpura, colitis, diabetes, psoriasis, pemphigus vulgaris, and rheumatoid arthritis.

20 13. A therapeutic composition according to claim 11, wherein the autoimmune disease is an arthritic condition.

14. A therapeutic composition according to claim 11, wherein the autoimmune disease is an inflammatory disease.

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15. A therapeutic composition according to claim 13, wherein the autoimmune disease is rheumatoid arthritis.

16. A therapeutic composition according to claim 12, wherein the

autoimmune disease is multiple sclerosis.

17. A synthetic peptide having an amino acid sequence comprising at least three residues selected from the group of amino acids consisting of aromatic acids,  
5 negatively charged amino acids, positively charged amino acids, and aliphatic amino acids, the synthetic peptide being at least seven amino acid residues in length and capable of binding to an MHC class II protein associated with an autoimmune disease.

18. A composition according to claim 17, wherein the aromatic amino acid is  
10 selected from the group consisting of tyrosine (Y), valine (V), and phenylalanine (F).

19. A composition according to claim 19, wherein the positively charged amino acid is lysine (K) and the sequence is selected from the group consisting of lysine-tyrosine (KY), lysine-valine (KV), and lysine-phenylalanine (KF).

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20. A composition according to claim 17, wherein the negatively charged amino acid is glutamic acid (E), and the sequence is selected from the group consisting of glutamic acid-lysine-tyrosine (EKY), glutamic acid-lysine-valine (EKV), and glutamic acid-lysine-phenylalanine (EKF).

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21. A composition according to claim 20, wherein the amino acid which is aliphatic is alanine (A), and the sequence is selected from the group consisting of glutamic acid-lysine-tyrosine-alanine (EKYA), glutamic acid-lysine-valine-alanine (EKVA), and glutamic acid-lysine-phenylalanine-alanine (EKFA).

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22. A composition according to claim 21 wherein the sequence further comprises an amino-terminal alanine, and the sequence is selected from the group consisting of alanine-glutamic acid-lysine-tyrosine-alanine (AEKYA), alanine-glutamic acid-lysine-valine-alanine (AEKVA), and alanine-glutamic acid-lysine-phenylalanine-

alanine (AEKFA).

23. A composition according to claim 22 wherein the autoimmune disease is an arthritic condition.

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24. A composition according to claim 23, wherein the arthritic condition is rheumatoid arthritis.

25. A composition according to claim 20, wherein the aliphatic amino acid is  
10 alanine, and the amino acid sequence is selected from the group consisting of: lysine-glutamic acid-tyrosine-alanine (KEYA), lysine-tyrosine-alanine-glutamic acid (KYAE), lysine-glutamic acid-valine-alanine (KEVA), lysine-valine-alanine-glutamic acid (KVAE), lysine-glutamic acid-phenylalanine-alanine (KEFA), and lysine-phenylalanine-alanine-glutamic acid (KFAE).

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26. A composition according to claim 19, wherein the aliphatic amino acid is alanine (A) and the amino acid sequence is selected from the group consisting of lysine-tyrosine-alanine-alanine (KYAA) or lysine-lysine-tyrosine-alanine (KKYA), lysine-valine-alanine-alanine (KVAA) or lysine-lysine-valine-alanine (KKVA), lysine-  
20 phenylalanine-alanine-alanine (KFAA), and lysine-lysine-phenylalanine-alanine (KKFA).

27. A composition according to claim 19, wherein the peptide further comprises two alanine residues, and the sequence is selected from the group consisting of alanine-lysine-tyrosine-alanine-glutamic acid (AKYAE), glutamic acid-alanine-lysine-  
25 tyrosine-alanine (EAKYA), alanine-lysine-valine-alanine-glutamic acid (AKVAE); and glutamic acid-alanine-lysine-valine-alanine (EAKVA), and alanine-lysine-phenylalanine-alanine-glutamic acid (AKFAE); and glutamic acid-alanine-lysine-phenylalanine-alanine (EAKFA).

28. A composition according to claim 17 wherein the peptide is 7-100 amino acid residues in length.

29. A composition which is a synthetic peptide having therapeutic activity in  
5 a subject suffering from an autoimmune disease, and the amino acid sequence having at least one of each of amino acids glutamic acid, lysine, and alanine and an amino acid selected from the group consisting of tyrosine, valine, and phenylalanine.

30. A composition according to claim 29 wherein the peptide is 7-100 amino  
10 acids in length.

31. A composition according to claim 30 wherein the peptide is 7-50 amino acids in length.

32. A composition according to claim 24 wherein the peptide is 7-25 amino  
15 acids in length.

33. A composition according to claim 32 wherein the peptide is 7-15 amino  
20 acids in length.

34. A composition according to claim 17 formulated as a unitary dosage in a pharmaceutically acceptable carrier.

35. A composition according to claim 17 which is substantially pure.  
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36. A composition according to claim 17 having greater affinity for the antigen binding groove of an MHC class II protein associated with the autoimmune disease than a type II collagen 261-273 peptide.

37. A composition according to claim 29 comprising amino acid analogs at residue locations and in amounts sufficient to inhibit protease degradation of the peptide in the subject.

5 38. An isolated peptide composition having a sequence selected from the group consisting of: AKEYAAAAAAKAAAA (SEQ ID NO: 7),  
AAEYAAAAAAKAAAA (SEQ ID NO: 12), AAKYAEAAAAKAAAA (SEQ ID NO: 15), and EAKYAAAAAAKAAAA (SEQ ID NO: 18).

10 39. An isolated peptide according to any of the peptides of claim 38, in which the tyrosine (Y) has been substituted by a valine (F) or a phenylalanine (F).

40. An isolated peptide composition having a sequence selected from the group consisting of: AEKYAAAAAAKAAAA (SEQ ID NO: 6),  
15 AKEYAAAAAAKAAAA (SEQ ID NO: 7), KEAYAAAAAAKAAAA (SEQ ID NO: 10),  
AAEYAAAAAAKAAAA (SEQ ID NO: 11), AAEYAAAAAAKAAAA (SEQ ID NO: 12), EKAYAAAAAAKAAAA (SEQ ID NO: 13), AAKYEAAAAAAKAAAA (SEQ ID NO: 14), AAKYAEAAAAKAAAA (SEQ ID NO: 15), EAAYAAAAAAKAAAA (SEQ ID NO: 16), EKKYAAAAAAKAAAA (SEQ ID NO: 17), EAKYAAAAAAKAAAA  
20 (SEQ ID NO: 18), AKKYEAAAAAAAAAAAA (SEQ ID NO: 21),  
AAEYKAAAAAAAAAAAA (SEQ ID NO: 26), AAKYEAAAAAAAAAAAA (SEQ ID NO: 28), AAKYAEAAAAAAAAAAAA (SEQ ID NO: 29), AEYAKAAAAAAAAAAAA (SEQ ID NO: 32), AEKAYAAAAAAAAAAAA (SEQ ID NO: 33), AYKAEAAAAAAAAAAAA (SEQ ID NO: 35), and AKYAEAAAAAAAAAAAA (SEQ ID NO: 36), the peptide having high  
25 affinity for an MHC class II protein.

41. An isolated peptide according to any of the sequences of claim 40, in which the tyrosine (Y) has been substituted by a valine (F) or a phenylalanine (F).

42. An isolated peptide composition having an amino acid sequence capable of inhibiting an immune response in a subject to an autoantigen, wherein a position in the amino acid sequence of the peptide that corresponds to an antigen binding pocket in a peptide binding groove of an MHC class II DR protein is identified as a particular amino acid.

43. An isolated peptide composition according to claim 42, wherein the autoantigen is associated with a condition selected from the group consisting of multiple sclerosis and arthritis.

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44. An isolated peptide composition according to claim 42, wherein the MHC class II protein is selected from the group consisting of an MHC class II HLA-DR1 protein, and an MHC class II HLA-DR4 protein.

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45. An isolated peptide composition according to claim 42, wherein the MHC class II protein is an MHC class II HLA-DR2 protein.

46. An isolated peptide composition according to claim 42, wherein the amino acid residue in the position of the sequence that corresponds to the P1 pocket in the MHC class II peptide binding groove is selected from the group consisting of a tyrosine, a valine, and a phenylalanine.

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47. An isolated peptide composition according to claim 42, wherein the amino acid residue in a first amino acid position of the sequence that corresponds to the P1 pocket in the MHC class II peptide binding groove is alanine.

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48. An isolated peptide composition according to claim 42, wherein the amino acid residue located eight residues beyond the first amino acid position of the sequence that corresponds to the P1 pocket in the MHC class II peptide binding groove is

selected from the group consisting of lysine and alanine residues.

49. A pharmaceutical preparation comprising a first peptide sequence and a second peptide sequence, wherein the preparation is a mixture of a first peptide and a second peptide of different amino acid sequences both according to claim 42 in a pharmaceutically acceptable carrier, the first sequence having in addition a lysine residue and the second sequence having an alanine residue at the amino acid position corresponding to eight residues beyond the amino acid corresponding to the P1 pocket in the MHC class II peptide binding groove.
50. A method of treating a subject having an autoimmune disease, comprising:
- (a) selecting a therapeutic agent comprising: a synthetic heteropolymer having at least two different amino acids, a first being an amino acid which is charged and a second being an amino acid which is hydrophobic, the amino acids being polymerized in a linear configuration, and a pharmaceutically acceptable carrier; and
  - (b) administering the therapeutic agent to the subject having the autoimmune disease.
51. A method according to claim 50, wherein in step (a) the charged amino acid is selected from the group consisting of lysine, glutamic acid, and aspartic acid.
52. A method according to claim 51, comprising in step (a) having a third amino acid which selected from the group of charged amino acids, the third amino acid being a different amino acid from the second amino acid.
53. A method according to claim 52, wherein the hydrophobic amino acid is selected from the group consisting of valine, leucine, isoleucine, alanine, phenylalanine, and tyrosine.



54. A method according to claim 53, wherein the heteropolymer comprises the amino acids lysine, alanine, and tyrosine.

55. A method according to claim 53, wherein the heteropolymer comprises  
5 the amino acids lysine, glutamic acid, alanine, and phenylalanine.

56. A method according to claim 53, wherein the heteropolymer comprises the amino acids lysine, glutamic acid, alanine, and valine.

10 57. A method according to claim 54, wherein the heteropolymer of step (a) contains lysine, alanine, and tyrosine polymerized in a molar ratio of at least 3 moles of lysine per mole of tyrosine, and at least 4 moles of alanine per mole of tyrosine.

58. A method according to claim 57, wherein the molar ratio of the lysine:  
15 alanine: tyrosine is 4.0: 5.0: 1.0.

59. A method according to claim 53, wherein the heteropolymer of step (a) contains the amino acids glutamic acid: lysine: alanine polymerized in a molar ratio of 1.5:  
4.0: 5.0.

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60. A method according to claim 51, wherein the heteropolymer contains the amino acids glutamic acid: alanine: tyrosine polymerized in a molar ratio of 1.5: 5.0: 1.0.

61. A method according to claim 51, wherein the heteropolymer contains the  
25 amino acids glutamic acid: lysine: tyrosine polymerized in a molar ratio of 1.5: 4.0: 1.0.

62. A method according to claim 50, wherein step (a) further comprises selecting the heteropolymer that inhibits binding of an antigenic peptide to an MHC class II protein.

63. A method according to claim 50, wherein step (a) further comprises selecting the heteropolymer that inhibits class II-specific T cell responses to an MHC class II protein-peptide complex.

5 64. A method according to claim 62, wherein the antigenic peptide is associated with an autoimmune disease.

65. A method according to claim 63, wherein the MHC class II protein is associated with an autoimmune disease.

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66. A method according to claim 50, wherein step (b) further comprises: supplementing the combined heteropolymer and carrier with at least an additional therapeutic agent.

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67. A method according to claim 66, wherein step (b) further comprises: selecting the additional therapeutic agent from the group consisting of an antibody, an enzyme inhibitor, an antibacterial agent, an antiviral agent, a steroid, a nonsteroidal anti-inflammatory agent, an antimetabolite, a cytokine, and a soluble cytokine receptor.

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68. A method according to claim 66, wherein step (b) further comprises: selecting an additional therapeutic agent that is an inducer of synthesis of a cytokine in a subject.

69. A method according to claim 67, wherein step (b) further comprises:  
25 selecting a cytokine from the group consisting of interferon- $\beta$ , interleukin-4 and interleukin-10.

70. A method according to claim 67, wherein step (b) further comprises: selecting an enzyme inhibitor from the group consisting of a protease inhibitor and a cyclooxygenase inhibitor.

71. A method according to claim 66, wherein step (a) further comprises:  
selecting the pharmaceutically acceptable carrier as suitable for administration to the  
subject by a route selected from the group consisting of intravenous, intramuscular,  
intraperitoneal, subcutaneous, oral, and transdermal administration.

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72. A method of making a therapeutic heteropolymer composition,  
comprising:

(a) selecting three or more amino acids from the group consisting of  
charged amino acids, aliphatic amino acids, and aromatic amino acids;

10 (b) polymerizing the amino acids selected in step (a) into a heteropolymer  
having a random linear configuration;

(c) obtaining the heteropolymer in step (b) that inhibits binding of an  
antigenic peptide to an MHC class II protein; and

(d) formulating in a pharmaceutically acceptable carrier, the  
15 heteropolymer that inhibits binding of an antigenic peptide to an MHC class II protein, to  
obtain a therapeutic heteropolymer composition.

73. A method according to claim 72, wherein step (c) further comprises:  
obtaining the heteropolymer that inhibits binding of an an antigen which is an autoantigen  
20 to an MHC class II protein.

74. A method according to claim 73, wherein the autoantigen in step (c) is  
collagen type II peptide 261-273 and the class II MHC protein is MHC HLA-DR1 or MHC  
HLA-DR4.

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75. A method according to claim 73, wherein the autoantigen in step (c) is  
myelin basic protein peptide 84-102 and the class II MHC protein is MHC HLA-DR2.

76. A method according to claim 74, wherein step (c) further comprises:

obtaining the heteropolymer that, at a concentration of 1.5 micromolar, inhibits 50% binding of collagen type II peptide 261-273, at a concentration of at least 20 micromolar, to a class II MHC HLA-DR1 or -DR4 protein.

5           77.           A method according to claim 75, wherein step (c) further comprises: obtaining the heteropolymer that, at a concentration of 1.5 micromolar, inhibits 50% binding of myelin basic protein peptide 84-102, at a concentration of at least 20 micromolar, to a class II MHC HLA-DR2 protein.

10           78.           A method according to claim 72, comprising prior to step (a) the additional step of producing the class II MHC protein recombinantly in a non-mammalian cell.

              79.           A method for obtaining an MHC class II binding motif amino acid  
15 sequence in a mixture of synthetic peptide heteropolymers having therapeutic activity in a subject, comprising:

                          (a) binding the mixture of synthetic heteropolymers to MHC class II protein molecules to form a heteropolymer-MHC protein complexes;

                          (b) removing by peptidase enzyme digestion the amino terminal amino  
20 acid residues of the heteropolymers protruding from the heteropolymer-MHC protein complex to align amino termini of the heteropolymers to the edge of the MHC protein complexes; and

                          (c) eluting the aligned heteropolymers from the MHC protein by dissociating the complexes to release the amino terminal aligned heteropolymers having  
25 the binding motif.

              80.           A method according to claim 79 wherein an additional step (d) comprises: determining the amino terminal sequence of the aligned heteropolymers, to identify the binding motif.

81. A method according to claim 80 wherein an additional step (e) comprises: comparing the amino terminal sequence of the aligned heteropolymers to the amino acid sequence of the synthetic heteropolymer composition.

5 82. A method according to claim 79, wherein the MHC class II protein is associated with an autoimmune disease.

83. A method according to claim 82, wherein the autoimmune disease is an arthritic condition or a demyelinating condition.

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84. A method according to claim 80, wherein an additional step (e) comprises: synthesizing a plurality of peptide preparations, each peptide preparation having a binding motif amino acid sequence.

15 85. A method according to claim 84 wherein an additional step (f) comprises: determining the affinity of each of the synthesized peptides for the MHC class II protein.

86. A method for treating a subject having an arthritic condition, comprising:  
obtaining a therapeutic composition which is a pure heteropolymer  
20 comprised of amino acids; and  
administering the composition in an effective dosage to the subject, such that the arthritic condition is remediated.

87. A method according to claim 86, wherein the effective dosage is at least  
25 5 mg per day.

88. A method according to claim 87, wherein the effective dosage is at least 10 mg per day.

89. A method according to claim 88, wherein the effective dosage is at least 15 mg per day.

90. A method according to claim 89, wherein the effective dosage is at least 20 mg per day.

91. A method according to claim 86, wherein the effective dosage is substantially in the range of 25 to 400  $\mu$ g per kg of the subject per day.

92. A kit for assaying the binding of an analyte to an MHC protein, comprising a water-soluble MHC protein which has been recombinantly produced in a non-mammalian cell, a reaction chamber for containing the analyte and the MHC protein, means for detecting binding of the analyte to the MHC protein, a container, and instructions for use.

93. A kit according to claim 92, wherein the MHC protein is an MHC class II protein selected from the group consisting of an MHC class II HLA-DR1, an MHC class II -DR2 and an MHC class II-DR4 proteins.

94. A kit according to claim 92, comprising in addition an autoantigenic peptide.